

# Nuclear Signaling in Smooth Muscle Cells Cyclic Nucleotide Phosphodiesterase 1A Moves In

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The second messenger cyclic GMP (cGMP) mediates signaling in the nervous system, genitourinary system, and the gastrointestinal tract. In the cardiovascular system, cGMP regulates vasorelaxation, vascular remodeling, platelet activation, and cardiac contractility. The ability of cGMP to regulate such a large number of temporally and spatially disparate processes is attributable to the many different isozymes that exist for synthesis and degradation of cGMP. These enzymes exhibit different subcellular localization and mechanisms of activation. Thus, cGMP is synthesized from GTP by both soluble and particulate forms of guanylyl cyclase. Soluble guanylyl cyclases are heterodimeric cytosolic enzymes that consist of  $\alpha$  and  $\beta$  subunits. There are several isoforms of the  $\alpha$  and  $\beta$  subunit,  $\alpha_1\beta_1$  being the most abundant combination in mammalian tissues.<sup>1–3</sup> Soluble guanylyl cyclase is activated by nitric oxide (NO) and NO-releasing drugs. Particulate forms of guanylyl cyclase, on the other hand, are transmembrane proteins that are activated by the natriuretic peptides ANP, BNP, and CNP.<sup>4</sup> Similarly, cGMP is degraded by a large number of cyclic nucleotide phosphodiesterases (PDEs). To date, 21 different PDE genes divided among 11 gene families have been identified in mammals. Most PDE families contain more than one gene, and most genes code for more than one mRNA. Although some PDE gene families hydrolyze cAMP exclusively (PDE4, PDE7, PDE8), many of these enzymes hydrolyze cGMP, or both cAMP and cGMP.<sup>5</sup> PDE5 is the major cGMP-specific PDE gene family in some cell types. Other cGMP-hydrolyzing PDE gene families include PDE1, PDE2, PDE6, PDE9, PDE10, PDE11, and possibly PDE3.<sup>6</sup> Any particular cell type typically expresses several different guanylyl cyclases and cGMP-hydrolyzing PDEs, which provide the cell with tools to precisely regulate cGMP synthesis and hydrolysis in different subcellular compartments and following exposure to different stimuli.

Many of the effects of cGMP are mediated by cGMP-dependent protein kinases.<sup>7</sup> The cGMP-dependent kinases (cGK or PKG) are serine/threonine kinase dimers encoded by two genes in mammals, cGKI and cGKII. Binding of cGMP to cGK induces a conformational change and exposure of the

catalytic center of the molecule, which is then able to phosphorylate a number of substrates in vivo. These substrates include ion channels, G proteins, cytoskeletal proteins, and transcriptional regulators.<sup>7–8</sup> In addition, cGMP exerts effects that are cGK-independent. For example, cGMP can regulate cAMP-dependent protein kinases (PKA) through direct or indirect mechanisms, as well as ion channels and guanine nucleotide exchange factors.

## cGMP PDEs Regulate Arterial Smooth Muscle Function, Possibly by Affecting Different Subcellular Pools of cGMP

In arterial smooth muscle cells (SMCs) a number of processes are controlled by cGMP. They include mechanical events that are regulated on a relatively rapid time scale. The contractile tone of the muscle is perhaps the best example of this.<sup>9</sup> Cyclic GMP causes smooth muscle relaxation in large part through its ability to lower intracellular calcium or activate myosin phosphatase.<sup>7</sup> Slower changes that are regulated by cGMP include gene transcription and altered proliferation in response to injury. As with all regulatory messengers, the amplitude and duration of the cGMP signals are governed by their rates of synthesis and rates of degradation.

The major cGMP PDEs present in arterial SMCs are PDEs1A, 1B, and 1C, and PDE5, although species differences exist for the PDE1 gene families.<sup>5</sup> Under basal low calcium conditions, the most active cGMP-hydrolyzing PDE is PDE5. The physiological importance of PDE5 in regulation of smooth muscle tone has been most effectively demonstrated by the successful clinical use of its selective inhibitors—sildenafil, tadalafil, and vardenafil—in the treatment of erectile dysfunction.<sup>10</sup>

Under conditions of increased levels of intracellular calcium (eg, during muscle contraction and in cells being stimulated with agents that result in increased intracellular calcium) one or more of the calcium-calmodulin activated PDE1 variants can become the predominant cGMP-hydrolyzing PDE. For example, in human aortic SMCs PDE5 is the predominant cGMP PDE under basal conditions, whereas in the presence of calcium-calmodulin PDE1C becomes a major contributor to cGMP hydrolysis.<sup>5</sup> Pharmacological approaches indicate that PDE1A may play a role in regulation of smooth muscle relaxation.<sup>11</sup> Vasoconstrictors, such as angiotensin II, cause an elevation of intracellular calcium levels that may lead to activation of PDE1A in vivo.<sup>11</sup> Interestingly, PDEs in the PDE1 gene family also appear to act as important regulators of SMC cell cycle progression and proliferation. In human SMCs, PDE1C expression is induced during cell cycle progression, and inhibition of PDE1C inhibits proliferation.<sup>12</sup> The study by

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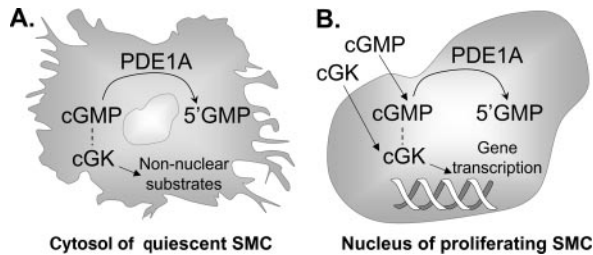
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Why does PDE1A translocate to the nucleus in synthetic proliferating arterial smooth muscle cells? A, In quiescent SMCs exposed to NO, cGMP is synthesized and subsequently activates cGMP-dependent protein kinase (cGK) and other cGMP effectors. Cytoplasmic PDE1A hydrolyzes cGMP, thereby reducing the activity of cGK and its ability to phosphorylate substrates located in the cytoplasm. B, When SMCs are stimulated to proliferate, PDE1A translocates to the nucleus, possibly together with cGK. In the nucleus cGK may stimulate gene transcription by phosphorylating transcriptional regulators. Hydrolysis of cGMP to 5'GMP could serve as a means to inactivate cGK, or prevent reactivation of cGK in the nucleus, thereby inhibiting its effect on gene transcription.

Nagel et al in this issue of *Circulation Research* addresses the role of PDE1A in SMCs.<sup>13</sup> The most significant observation is that PDE1A is localized to the cytoplasm of quiescent SMCs but translocates to the nucleus in synthetic and proliferating SMCs (see Figure). In quiescent rat SMCs, inhibition of PDE1A resulted in inhibition of myosin light chain phosphorylation. Inhibition of PDE1A in proliferating SMCs, on the other hand, resulted in inhibition of proliferation and induction of apoptosis. Thus, cytoplasmic PDE1A and nuclear PDE1A appear to serve different functions in SMCs, most likely by affecting different pools of cGMP. These studies add PDE1A to the growing number of cytoplasmic signaling molecules that can also have important nuclear functions, and imply that cGMP has essential signaling functions in the nucleus that mediate the growth-inhibitory and proapoptotic effects of cGMP in SMCs. In this context it is interesting to note that in some studies cGMP–cGK promotes proliferation of primary SMCs in culture and of SMCs in vivo, but inhibits proliferation of subcultured SMCs.<sup>14–15</sup> It is tempting to speculate that the discrepancies in the literature on the antiproliferative effects of cGMP can be explained in part by distinct downstream mediators of different subcellular pools of cGMP.

### What Is PDE1A Doing in the Nucleus?

Although it is now quite well established that cAMP can be spatially restricted to different microdomains or pools in the cell,<sup>16</sup> less is known about subcellular compartmentalization of cGMP. Experiments using cAMP-sensitive fluorescence resonance energy transfer (FRET) indicators have shown that cAMP levels can be elevated in the nucleus as soon as 2 to 3 minutes after agonist stimulation of membrane receptors.<sup>17</sup> Interestingly, in these studies cAMP was shown to diffuse into the nucleus well before the catalytic subunit of PKA, suggesting that free cAMP diffuses to the nuclear compartment without accompanying PKA. It is likely that free cGMP would also diffuse into the nucleus through a similar mechanism. In addition, there are a few reports of nuclear translocation of cGK after cGMP binding.<sup>18–19</sup> Nuclear cGK

might physically interact with, and regulate, transcriptional regulators, such as TFII-I.<sup>20</sup> The nuclear localization of a cGMP PDE reported by Nagel and colleagues is not unprecedented. Another cGMP PDE (PDE9A) also appears to localize primarily to the nucleus,<sup>21</sup> suggesting that precise regulation of nuclear cGMP levels is important for proper cell function.

More detailed studies are required to increase our understanding of the role of nuclear cGMP levels and cGMP PDEs. For now, we are left with many intriguing questions. For example, is cGMP synthesized in the nucleus, as suggested by early studies on hepatocytes,<sup>22</sup> or does it diffuse from the cytosol or translocate there bound to cGK? How do nuclear cGMP levels regulate cell cycle progression and apoptosis? What are the nuclear substrates of cGK? How does PDE1A get in and out of the nucleus? Is the role of nuclear PDE1A to prevent nuclear cGK from being reactivated, or is PDE1A able to hydrolyze cGMP bound to cGK or other cGMP effectors? Some of these questions can now be addressed by using fluorescent indicators that report subcellular localization of cyclic nucleotide levels.<sup>17,23</sup> Such FRET indicators for monitoring intracellular levels of cGMP have been generated,<sup>24,25</sup> most recently using fusion proteins based on the regulatory GAF domain from PDE5A.<sup>23</sup> It is expected that the next few years will reveal more details in the regulation and downstream effects of nuclear cGMP signaling in SMCs and other cell types.

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